

Appl. No. 09/937,452
Amtd. Dated September 7, 2004
Reply to Office Action of May 5, 2004

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (currently amended) A method for the in vitro micropropagation and phytofortification of a phytopharmaceutical plant comprising:
 - a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising at least one plant growth regulator having cytokinin activity, to form regenerated tissue;
 - b) transferring said regenerated tissue to a basal medium lacking said plant growth regulator having cytokinin activity, and culturing to form plantlets; and
 - c) subculturing said plantlets onto a basal medium supplemented with at least one, or more than one additive of interest at an amount from about 50 to about 200 mg/L, said additive of interest selected from the group consisting of a vitamin, boron, chromium, cobalt, copper, iron, lithium, iodine, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc, to allow uptake and accumulation of said at least one, or more than one additive of interest in a bioavailable form in said plantlet thereby producing said phytopharmaceutical plant that is phytofortified.
2. (previously presented) The method of claim 1, wherein after said step of culturing (step a)), and prior to said step of transferring (step b)), said regenerated tissue is placed on a basal medium and subcultured to further formation of regenerated tissue.
3. (currently amended) The method of claim 1 wherein after said step of transferring (step b)), said plantlet is transferred to a hydroponic environment with a recycling solution containing at least one, said one or more than one additive of interest to allow uptake and accumulation of said at least one, or more than one additive of interest in a bioavailable form within said plantlet or seedling.

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4-6. (canceled)

7. (previously presented) The method according to claim 1, wherein said at one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (N-phenyl-N'-(1,2,3-thidiazol-yl)urea), benzylaminopurine(BAP), zeatin, CPPU (N-(2-chloro-4pyridyl)-N(-phenyl urea) and 2-I-P (N6-(2-isopentenyl) adenine).

8. (currently amended) The method according to claim 7, wherein said at least one, or more than one plant growth regulator having cytokinin activity is selected from thidiazuron (TDZ) and benzylaminopurine (BAP).

9. (currently amended) The method according to claim 8, wherein said induction medium comprises from about 0.001 to about 25 $\mu\text{mol}\cdot\text{L}^{-1}$ of said at least one, or more than one plant growth regulator having cytokinin activity.

10. (original) The method according to claim 8, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.

11. (previously presented) The method according to claim 1, wherein said explant is selected from the seed, petiole, hypocotyl, stem, cotyledon and leaf.

12. (previously presented) The method according to claim 1, wherein said phytopharmaceutical plant is St. John's wort.

13. (original) The method according to claim 12, wherein said plant growth regulator having cytokinin activity is thidiazuron.

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14. (original) The method according to claim 13, wherein the induction medium comprises thiadiazuron from about 0.001 to about 25 p.mol •L-1.

15. (original) The method according to claim 14, wherein the induction medium comprises thiadiazuron from about 4 to about 10 μ mol •L-1.

16. (original) The method according to claim 12, wherein said sterile explant is maintained on said induction medium from about 1 to about 15 days.

17. (original) The method according to claim 16, wherein said sterile explant is maintained on said induction medium from about 8 to about 10 days.

18. (original) The method according to claim 12, wherein said explant is etiolated hypocotyl.

19-39. (canceled)

40. (currently amended) The method according to claim 4, wherein said at least one, or more than one additive of interest is zinc.

41. (canceled)

42. (currently amended) The method according to claim 4, wherein said at least one, or more than one additive of interest within said basal medium, is from about 0.001 to about 500 mg.L-1.

43. (previously presented) The method according to claim 2, wherein, in said transferring step, said regenerated tissue is subcultured for about 1 to about 15 days.

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44. (currently amended) A method for phytofortification of an in vitro-grown phytopharmaceutical plant comprising:

- a) culturing a sterile seedling, explant or regenerated tissues to form a plantlet; and
- b) subculturing said plantlet onto a basal medium lacking a plant growth regulator having cytokinin activity, said basal medium supplemented with at least one, or more than one additive of interest, at an amount from about 50 to about 200 mg/L, said additive of interest selected from the group consisting of a vitamin, boron, chromium, cobalt, copper, iron, lithium, iodine, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc, to allow uptake and accumulation of said at least one, or more than one additive of interest in a bio-available form in said plantlet to produce a phytofortified phytopharmaceutical plant.

45. (currently amended) The method according to claim 44, wherein, in said step of culturing, said plantlets are produced either:

- a) on a sterile explant of said phytopharmaceutical plant grown on an induction medium comprising at least one, or more than one plant growth regulator having cytokinin activity, or
- b) grown from a sterile seed, or
- c) seedling in culture.

46. (previously presented) The method according to claim 45, wherein said at one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (N-phenyl-N'-(1,2,3-thidiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (N-(2-chloro-4-pyridyl)-N(-phenyl urea) and 2-i-P (N6-(2-isopentenyl) adenine).

47. (currently amended) A phytopharmaceutical plant prepared by the method of claim 1 and comprising an elevated level of said one or more than one additive of interest introduced in the said step of subculturing (step c)), when compared to a plant grown in said basal medium in the

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absence of said additive of interest.

48. (previously presented) A method for promoting shoot formation of a phytopharmaceutical plant comprising the steps of:

- a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising one or more plant growth regulators having cytokinin activity, to form regenerated tissue; and
- b) transferring said regenerated tissue to a basal medium lacking said plant growth regulator having cytokinin activity and culturing to form plantlets, and wherein said steps of culturing and transferring result in the in vitro micropropagation involving de novo shoot formation of non-meristematic tissue of said phytopharmaceutical plant.

49. (currently amended) A method for the in vitro micropropagation and phytofortification of a phytopharmaceutical plant comprising:

- a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising at least one, or more than one plant growth regulator having cytokinin activity, to form regenerated tissue;
- b) transferring said regenerated tissue to a basal medium and culturing to form plantlets; and
- c) subculturing said plantlets onto a basal medium containing an additive selected from the group consisting of lithium, chromium, nickel, selenium, silicon, tin, and vanadium, to allow uptake and accumulation of said lithium additive in a bio-available form in said plantlet, said basal medium lacking said plant growth regulator having cytokinin activity.